CLAIMS

1. A rat embryonic stem cell characterized by having the following properties (a)-(j):

(a) expressing Oct3/4 gene and Nanog gene,

(b) positive for alkaline phosphatase activity,

(c) having an embryoid body forming ability,

(d) expressing SSEA (Stage-Specific Embryonic Antigen)-1 and SSEA-4,

(e) having the same number of chromosomes as does a normal rat cell,

(f) capable of being subcultured and holding the undifferentiated state,

(g) having *in vitro* pluripotency,

(h) having a potential to differentiate for cells of three embryonic germ lineages,

(i) having teratoma formation ability,

(j) having an ability to produce a chimeric rat.

2. A rat embryonic stem cell obtained by performing a process comprising the following steps (A)-(D), under the culture conditions using a substantially serum free culture medium:

(A) a step for dissociating an inner cell mass formed by the culture of rat blastocysts, remaining a state of cell aggregate,

(B) a step for culturing primary embryonic stem cells resulting from the culture of the dissociated inner cell mass until it can be passaged,

(C) a step for dissociating the primary embryonic stem cells, which have become capable of being passaged, remaining a state of cell aggregate, followed by passaging and culturing the same,

(D) a step for further passaging and culturing the cells to establish an embryonic stem cell.

3. The embryonic stem cell of claim 2, wherein the culture medium comprises a serum replacement reagent.

4. The embryonic stem cell of claim 2 or 3, wherein the step (A) comprises a step for mechanically dissociating the inner cell mass.

5. The embryonic stem cell of any of claims 2 to 4, wherein the step (C) comprises a step for mechanically dissociating the embryonic stem cells.

6. The embryonic stem cell of any of claims 2 to 5, wherein a culture medium without rat leukemia inhibitory factor (rLIF) is used in step (A).

7. The embryonic stem cell of any of claims 2 to 6, wherein an rLIF-containing culture medium is used in steps (B)-(D).

8. A production method of a rat embryonic stem cell which comprises performing a process comprising the following steps (A)-(D), under the culture conditions using a substantially serum free culture medium:

(A) a step for dissociating an inner cell mass formed by the culture of rat blastocysts, remaining a state of cell aggregate,

(B) a step for culturing primary embryonic stem cells resulting from the culture of the dissociated inner cell mass until it can be passaged,

(C) a step for dissociating the primary embryonic stem cells, which have become capable of being passaged, remaining a state of cell aggregate, followed by passaging and culturing the same,

(D) a step for further passaging and culturing the cells to establish an embryonic stem cell.

9. The production method of claim 8, wherein the culture medium comprises a serum replacement reagent.

10. The production method of claim 8 or 9, wherein the step (A) comprises a step for mechanically dissociating the inner cell mass.

11. The production method of any of claims 8 to 10, wherein the step (C) comprises a step for mechanically dissociating the embryonic stem cells.

12. The production method of any of claims 8 to 11, wherein an rLIF-free culture medium is used in step (A).

13. The production method of any of claims 8 to 12, wherein an rLIF-containing culture medium is used in steps (B)-(D).

14. A subculture method of rat embryonic stem cells which comprises dissociating and passaging the cells, remaining a state of cell aggregate.

15. The subculture method of claim 14, which comprises a step for mechanically dissociating the cells.

16. The subculture method of claim 14 or 15, wherein the cells are cultured using a substantially serum free culture medium.

17. The subculture method of claim 16, wherein the culture medium comprises a serum replacement reagent.

18. The subculture method of claim 16 or 17, wherein the culture medium comprises rLIF.

19. A culture medium for rat embryonic stem cell, which comprises a serum replacement reagent and rLIF.

20. A culture kit for rat embryonic stem cell, which comprises a serum replacement reagent and rLIF as components.

21. The culture kit of claim 31, which further comprises the rat embryonic stem cell of any of claims 1 to 7 as a component.

22. The culture kit of claim 20 or 21, which further comprises feeder cells as a component.

23. The culture kit of claim 22, wherein the feeder cells are embryo-derived normal fibroblasts.

24. A differentiation induction method of a rat embryonic stem cell, which comprises stimulating the rat embryonic stem cell of any of claims 1 to 7 with a differentiation inducer.

25. The differentiation induction method of claim 24, wherein the differentiation inducer is a retinoic acid, growth factor, glucocorticoid or extracellular substrate.

26. A cell obtained by inducing the differentiation of the rat embryonic stem cell of any of claims 1 to 7.

27. A cDNA library, genomic library or cell extract derived from the rat embryonic stem cell of any of claims 1 to 7.

28. A screening method of a differentiation inducer for tissue or cell, which comprises the following steps (i)-(iii):
(i) a step for bringing a test substance into contact with the rat embryonic stem cell of any of claims 1 to 7,
(ii) a step for evaluating the presence or absence or the extent of the differentiation of the rat embryonic stem cell,
(iii) a step for judging whether or not the test substance is a substance associated with differentiation induction, based

on the evaluation results of the above-mentioned (ii).

29. A screening method of a substance acting on the differentiation induction of tissue or cell, which comprises the following steps (I)-(III):

(I) a step for bringing a test substance into contact with the rat embryonic stem cell of any of claims 1 to 7,

(II) a step for culturing the rat embryonic stem cell of the aforementioned (I) under the conditions allowing differentiation induction of the embryonic stem cell, and evaluating the presence or absence or the extent of the differentiation thereof,

(III) a step for judging whether or not the test substance is a substance acting on the differentiation induction of tissue or cell, based on the evaluation results of the above-mentioned (II).

30. A use of the rat embryonic stem cell of any of claims 1 to 7 in the production of a genetically modified rat.

31. The use of claim 30, wherein the genetically modified rat is either of a chimeric rat, knockout rat, knockin rat, transgenic rat and knockdown rat.

32. A production method of a genetically modified rat, which comprises performing a process comprising the following steps (X)-(Z):

(X) a step for introducing a desired gene into the rat embryonic stem cell of any of claims 1 to 7,

(Y) a step for preparing an oocyte for transplantation comprising the rat embryonic stem cell into which the gene was introduced,

(Z) a step for transferring the oocyte for transplantation into a pseudopregnant female rat to produce an offspring rat.

33. A genetically modified rat produced by the production method of claim 32.

34. The rat of claim 33, which is either of a chimeric rat, knockout rat, knockin rat, transgenic rat and knockdown rat.